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Case Report and Treatment of Hole in the Head in Oscar, *Astronotus ocellatus*

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Abstract

Hole in the head is one of the important diseases of cichlid fishes that caused by Heximita or Spironucleus infections. In December 2007, a diseased Oscar was referred to the veterinary hospital, Shahid Chamran University, Ahvaz, Iran. Infected fish showed an initial loss of appetite, exophthalmia, darkness of skin followed by holes appearing on the head surface and a complete refusal to feed. For detecting the hexamitid flagellates, investigation was undertaken by taking the smear from the holes in head. Heavy Flagellate infection was detected in the smears and the parasite recognized as hexamita according to the shape, type of movement and size of the parasite with photomicroscopy directly and examination of tissue squash in Giemsa-stained dry smear preparations. In order to detect the bacterial infection of the lesions, the sample was taken from the holes and cultured on TSA and kept at 25°C for 24 h. Large number of *Aeromonas hydrophila* colonies were appeared on the agar after 24 h and recognized by bacteriological tests. The fish was treated with long term bath of 6 mg/l metronidazole and after 24 h, with 0.2 ppm malachite green mixed with 0.15 ppm formalin. The fish fed a balanced and vitamin enriched diet by stomach tube for several days. The fish gradually showed signs of improvement and after 10 days, the fish completely recovered.

Keywords: Oscar, Heximita, *Aeromonas hydrophila*, metronidazole

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Introduction

Hole in the head, also known as head and lateral line erosion, is one of the important diseases of cichlid fishes that caused by *Heximita* or *Spironucleus* infections. The disease has been reported from many tropical species including freshwater and marine fishes in *Cichlidae, Anabantidae, Belontidae, Acanthuridae and Pomacentridae* (Bassleer 1983, Gratzek 1988). Severe systemic infection by a diplomonad flagellate, *Spironucleus barkhanus* (Hexamitidae) was also reported in postsmolts and adult salmon cultured in seawater cages in Norway and Canada (Guo and Woo, 2004). The causative parasites are commonly recorded from the digestive tracts of cichlids in addition to cyprinids, eels and salmonids and have been recorded from a wide range of internal organs within these hosts including heart, kidney, liver, spleen, eyes, brain and blood (Paull and Matthews, 2001). Although hexamita infestations are a major problem in aquarium fish industry, reliable data on drug treatment patterns or on the treatment methods of hexamita infestations are not available.

Metronidazole is the only member of 5-nitroimidazole group approved for using in the United States in human and it is the drug of choice for treating giardiasis and amoebiasis (mainly refer to the disease caused by *Entamoeba histolytica*) in many countries (Campanati and Monteiro-Leal, 2001). Both giardiasis and amoebiasis causative agents are anaerobic endoparasites, and act similar to hexamita thus this parasitic disease might also respond to metronidazole treatment. In this study the anti-protozoal effect of metronidazole was examined on the treatment of naturally infected Oscar fish.

Case Presentation

In December 2007, a diseased Oscar was referred to the veterinary hospital, Shahid Chamran University, Ahvaz, Iran. Infected fish showed an initial loss of appetite, exophthalmia, darkness of skin followed by holes appearing on the head surface and a complete refusal to feed (Fig. 1). For detecting the hexamitid flagellates, investigation was undertaken by taking the smear from the holes in head.

In order to detect the bacterial infection of the lesions, the sample was taken from the holes. For bacterial identification, TSA medium was used for primary isolation. The medium was incubated at room temperature (approximately 25ºC). Grown colonies were selected on the basis of shape, arrangement, staining characteristics, oxidase and catalase activity, and then streaked to purity on new plates. Standard bacteriological methods were used for identification including oxidation/fermentation, methyl red, phenylalanine deaminase, starch hydrolysis, gelatin liquefaction, and carbohydrate utilization. The growing ability at 4, 15 and 37º C was determined by incubating of TSA and TCBS for one week.

Results and Discussion

Heavy flagellate infection was detected in the smears and the parasite recognized as Hexamita according to the shape, type of movement and size of the parasite with the photo microscopy and examination of tissue squash preparations in Giemsa-stained smear preparations. Also large numbers of *Aeromonas hydrophila* colonies were appeared on the agar after 24 h and recognized by bacteriological tests. Aeromonads are characteristic bacteria from an aquatic environment. The motile Aeromonads species are often ubiquitous members of the aquatic ecosystem, but all can be components of the microbial flora of aquatic animals and may be pathogens of poikilotherms, homoiotherms and even humans. *A. hydrophila, A. caviae* and *A. sobria* have all been isolated from fish with the clinical signs of bacterial septicemia. Although it has been reported that water temperature and other environmental factors such as crowding and handling, as well as low dissolved oxygen and nitrate levels may be

involved in the disease process. The exact pathogenic mechanism of aeromonads is unknown.

The affected fish was treated with long term bath of 6 mg/l metronidazole and after 24 hours, with 0.2 ppm malachite green mixed with 0.15 ppm formalin. The fish fed a balanced and vitamin enriched diet by stomach tube for several days (crushed sheep liver and multivitamin syrup). The fish gradually showed signs of improvement and after 10 days, the fish completely recovered (Fig. 2).

In this study, metronidazole had a significant effect on Hexamita infection. Metronidazole is used to treat a broad range of infections caused by anaerobic parasites and bacteria. This drug is also effective against human amoebiasis (Land and Johnson, 1997). Metronidazole appeares to have a role in inducing DNA damage and DNA breakage due to treatment in human lymphocytes through the futile cycle. Metronidazole is the only 5-nitroimidazole approved for used in the United States and it is the drug of choice for treating giardiasis and amoebiasis (mainly refer to disease caused by *Entamoeba histolytica*) in many countries (Campanati and Monteiro-Leal, 2001). The selective cytotoxicity of metronidazole relies on biochemical properties of anaerobic organisms that are lacking in the aerobic cells. For the aerobic cells, the inhibitory concentration of metronidazole is two to three orders of magnitude higher then the anaerobes (Land, and Johnson, 1997). The one-electron reduction of the drugs leads to the production of nitro radical anions. In the presence of oxygen, these radicals are reoxidized and generate oxygen-activated species that can be responsible for the DNA and other cell component damages. (Menéndez *et al.,* 2001; Re *et al.,* 1997).

Both giardiasis and amoebiasis causative agents are anaerobic endoparasite and act similar to Hexamita. Therefore, Hexamitosis appeares also respond well to metronidazole treatment.

In conclusion, it was shown that, long term bath of metronidazole in concentration of 6 mg/l followed by treatment with malachite green and formalin mixture, could be an effective treatment of hole in the head. The authors believe this is the first reported case of using metronidazole plus malachite green and formalin for the treatment of hole in the head in Iran.



Figure 1: Exophthalmia associated with holes in the frontal region.



Figure 2: Recovered fish after 10 days.

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گزارش بیماری سوراخ شدن سر (Hole in the Head) در ماهی اسکار زینتی و درمان آن

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چکیده

بیماری سوراخ شدن سر یکی از بیماریهای تک‌یاخته‌ای مهم و کشنده در انواع ماهیان پرورشی و آکواریومی می‌باشد که عامل آن هگزامیتا و اسپیرونکلئوس می باشد. در تاریخ ۱۳۸۶/۶/۲۵ ماهی اسکار زینتی توسط یکی از مراکز فروش ماهیان آکواریومی اهواز به بیمارستان دامپزشکی ارجاع داده شد. علائم قابل مشاهده بیماری شامل دپرسیون شدید، بی‌اشتهایی، تغییررنگ و تیره شدن بدن، وجود سوراخ‌ها و زخم‌های چرکی در سر و بیرون‌زدگی شدید چشم‌ها بود. جهت تشخیص، نمونه‌گیری با کتریایی و کشت مرطوب از حفرات سر، انجام شد. در گسترش مرطوب آلودگی شدید به تک‌یاخته هگزامیتا مشاهده شد. در کشت باکتریایی بر روی محیط TSA از سوراخهای سر و نگهداری در دمای ۲۵ درجه سانتیگراد، آلودگی شدید به باکتری آئروموناس هیدروفیلا مشخص گردید. بلافاصله پس از تشخیص بیماری، ماهی تحت درمان قرار گرفته و به مدت ۱۰ روز در بیمارستان دانشکده دامپزشکی بستری شد. جهت درمان بیماری هگزامیتوز از حمام طولانی مدت مترونیدازول با غلظت mg/L ۶ بصورت محلول در آب استفاده گردید. پس از ۲۴ ساعت جهت رفع عفونتهای ثانویه حمام طولانی مدت مالاشیت گرین با غظت mg/L ۰/۲ به همراه فرمالین با غلظت ppm ۰/۱۵ استفاده شد. جهت تأمین مواد غذایی و به علت بی‌اشتهایی ماهی در این مدت، ماهی با استفاده از لوله دهانی با غذای مایع تغذیه می‌شد. چند روز پس از درمان، ماهی علائم بهبود را نشان داد و وضعیت شنا و فعالیت ماهی پس از ده روز به حالت طبیعی برگشت.

واژگان کلیدی: ماهی اسکار، هگزامیتا، آئروموناس هیدروفیلا، مترونیدازول